Claims

1. Method for the realization of a confocal fluorescence in vivo in situ image, the method using an image guide made of several thousands of optical fibres and consisting of the point by point scanning of a tissue in a subsurface plane, each point corresponding to an excitation signal emitted by a continuous source, deflected and injected into one of the optical fibres of said bundle then focussed on the exit of said fibre in said plane, each point emitting in return a fluorescence signal collected by said optical fibre, then detected and digitized to form an image element, characterized in that the excitation signal is deflected at a speed corresponding to acquisition of a number of images per second sufficient for a real-time use and in that the fluorescence signal is detected at a detection frequency corresponding to a minimum sampling frequency of the fibres one-by-one.

2. Method according to claim 1, characterized by a numerical aperture of the focussing optics between approximately 0.5 and 1.

3. Method for the realization of a high-resolution fluorescence image using an image guide made of several thousands of optical fibres, an excitation signal being emitted by a continuous source, deflected and injected by turns into one of the optical fibres of said image guide and a fluorescence signal emitted in response being collected by the same optical fibre as that used for the excitation, then detected and digitized to form an image element, characterized in that the end of the fibres is intended to be placed bare directly in contact with the surface of the tissue to be imaged, each fibre being able to produce a divergent beam which is able to excite a microvolume of tissue situated at the surface to a maximum depth depending in particular on the core diameter of the optical fibres and in that the excitation signal is deflected at a speed corresponding to acquisition of a number of images per second sufficient for a real-time use and in that the fluorescence signal

is detected at a detection frequency corresponding to a minimum sampling frequency of the fibres one-by-one.

4. Method according to one of the preceding claims, characterized in that the deflection speed of the excitation beam is adjusted by determining a rapid-resonance frequency of a resonating line mirror and a slow-resonance frequency of a galvanometric frame mirror.

5. Method according to one of the preceding claims, characterized in that optical deflection, injection, focussing and detection means are used having a degree of achromaticity which allows the collection of photons over the whole of the emission band of the excited fluorophore.

6. Method according to one of the preceding claims, characterized by a quantum efficiency of detection at the fluorescence wavelengths to be detected of at least 50 %.

7. Method according to one of the preceding claims, characterized by a prior step of detecting the placement of the fibres of the image guide which are intended to be used.

8. Method according to one of the preceding claims characterized by a prior step of determining the real injection rate particular to each fibre.

9. Method according to one of the preceding claims, characterized by a prior step of determining the collected flux corresponding to the background image.

10. Method according to claims 8 and 9, characterized by a step of correcting the digitized signal coming from a fibre by subtraction of the flux corresponding to the background image and adaptation to the real rate of injection which is particular to said fibre.

11. Method according to claim 10, characterized by a step of reconstructing the image from the corrected signal.

12. Method according to claim 11, characterized in that the step of reconstructing the image comprises a Gaussian low-pass filtering.

13. Apparatus for in situ in vivo fibred optic confocal fluorescence imaging for the implementation of the method according to one of claims 1, 2, 4-12, comprising:

- the image guide (6);
- the source (1) emitting continuously at the excitation wavelength of at least one targeted fluorophore,
- means for rapid scanning (4) and injection (5) fibre by fibre over time of the excitation beam produced by the source (1) by lines and by columns in a XY plane corresponding to the entry section of the image guide (6);
- means (3) for separating the excitation wavelength and the fluorescence wavelengths;
- means for detection (11) of the fluorescence signal; and
- means (12) for processing the detected signal allowing the realization of an image;

an optical head (7) being arranged at the distal end, intended to be brought into contact with the observed tissue (13), allowing the excitation beam to be focussed.

characterized in that:

- the scanning means are suitable for moving the excitation beam at a speed corresponding to the obtaining of an image in real time; and
- the detection means have a pass-band whose frequency is fixed as a function of the minimum one-by-one fibres sampling frequency.

14. Apparatus for in situ in vivo fibred high-resolution confocal fluorescence imaging for the implementation of the method according to one of claims 3-12, comprising:

- the image guide (6);
- the source (1) emitting continuously at the excitation wavelength of at least one targeted fluorophore,

- means for rapid scanning (4) and fibre-by-fibre injection (5) of the excitation beam produced by the source (1) in a XY plane corresponding to the entry section of the image guide (6);
- means (3) for separating the excitation wavelength and the fluorescence wavelengths;
- means (11) for detecting the fluorescence signal; and
- means (12) for processing the detected signal allowing the realization of an image;

characterized in that the end of each fibre is adapted for producing a beam which is divergent and is intended to be placed bare directly in contact with the surface of the tissue to be observed;

and in that the scanning means are suitable for moving the excitation beam at a speed corresponding to the obtaining of an image in real time; and the detection means have a pass-band whose frequency is fixed as a function of the minimum sampling frequency of the fibres one-by-one.

15. Apparatus according to claim 13 or 14, characterized in that the excitation beam produced by the source (1) is of the longitudinal monomode type presenting an optimum wave front quality for the injection into a slightly multimode optical fibre.

16. Apparatus according to one of claims 13 to 15, characterized in that, the section of a fibre being circular, the excitation beam produced by the source is circular so as to optimize the injection into a fibre.

17. Apparatus according to one of claims 13 to 16, characterized by means (2) for shaping the beam used on the exit of the source (1) in order to shape the excitation beam so as to adapt it to the injection means (5) in the image guide (6).

18. Apparatus according to one of claims 13 to 17, characterized in that the means for separating the excitation and fluorescence wavelengths comprise a dichroic filter (3) having a maximum efficiency at the excitation wavelength.

19. Apparatus according to one of claims 13 to 18, characterized by rejection means (8) placed upstream of the detection means (11) and suitable for eliminating the excitation wavelength.

20. Apparatus according to one of claims 13 to 19, characterized in that the scanning means (4) comprise a resonating line mirror (M1), a galvanometric frame mirror (M2), a first afocal optical system (AF1) with unitary magnification adapted for the conjugation of the two mirrors and a second afocal system (AF2) with unitary magnification adapted for the conjugation of the rotation planes of the two mirrors with the injection plane in one of the fibres.

21. Apparatus according to one of claims 13 to 20, characterized in that the optical means of the optical head (7), the scanning means (4), the injection means (5) and the detection means present a degree of achromaticity adapted for the collection of the photons over the whole of the width of the spectral band of the fluorescence signal.

22. Apparatus according to one of claims 13 to 22, characterized in that the injection means (5) comprise two optical units (E1, E2), the first unit (E1) being adapted for correcting the optical aberrations at the edge of the field of the scanning means (4) and the second unit (E2) being adapted for carrying out the actual injection in one of the fibres of the image guide (6).

23. Apparatus according to claim 22, characterized in that the first optical unit (E1) comprises a doublet and the second optical unit (E2) comprises two doublets followed by a lens.

24. Apparatus according to one of claims 13 to 23, characterized by a filtering hole (10) placed in front of the detection means (11) whose diameter is chosen so that the image of a fibre fits into it.

25. Apparatus according to claim 24, characterized by means (9) for focussing the fluorescence signal on the filtering hole (10).